Photosynthetic carbon metabolism and photorespiration

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ACCLIMATION OF THE MARINE DIATOM *Phaeodactylum tricornutum* FROM HIGH CO_2 TO LOW CO_2

TAKUMI HARA & YUSUKE MATSUDA
Department of Chemistry, Kwansei-Gakuin Univ., Nishinomiya, 662-8501

Changes in the photosynthetic affinities for dissolved inorganic carbon (DIC) in the marine diatom, *Phaeodactylum tricornutum* (UTEX640) during acclimation from high CO_2 to low CO_2 were monitored. The measurement was done with Clark-type O_2 electrode and it was found that cells gown in 5%-CO_2-enriched air exhibited affinity for DIC 10% that of cells grown in atmospheric CO_2.

The induction of the High-affinity photosynthesis for external DIC started immediately after switching the cells from high CO_2 to air, and completed in the next 24hrs. External carbonic anhydrase (CA) activity was not detected throughout the acclimation. The addition of bovine erythrocyte CA to the assay system stimulated photosynthetic affinity during the early acclimation stages. Such stimulation however was not observed in the cells totally acclimated to low CO_2. The light dependency of the acclimation of high-CO_2 grown cells to low CO_2 was also examined.

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Purification and characterisation of soluble carbonic anhydrase from the marine diatom *Phaeodactylum tricornutum*.

Yasutaka HIRAOKA, Dan SATO, Yusuke MATSUDA
Department of Chemistry, Kwansei-Gakuin Univ., Nishinomiya 662-8501.

Microalgae, in general, are thought to possess the inorganic carbon concentrating mechanism (CCM). This is supposed to be one of the crucial mechanisms to accomplish high primary productivity in the aquatic environment. The mechanistic bases of CCM in marine diatoms are not studied extensively, even though they are considered to be one of the major photoautotrophs in the ocean. Carbonic anhydrase (CA, EC 4.2.1.1) is believed to be one of the important components of the CCM. In this study, CA was purified from the marine diatom *Phaeodactylum tricornutum* (UTEX642) and partially characterized. In air-grown cells, there was little extracellular CA activity detected whereas that in the supernatant of the cell lysate was high. CA activity was fractionated by salting out with ammonium sulfate and followed by column chromatography using DEAE-sephacel and *p*-aminomethylbenzene sulfonamide-agarose. The resulting CA fraction was comprised of elecrophoretically-single protein. The purified CA was shown in size at 29kDa. A physiological assay suggested that this CA was induced in the soluble fraction of the cells in response to decrease in the inflow [CO2] in the media.

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Acclimation of CO_2-insensitive mutant of the green alga *Chlorella ellipsoidea* from air to high CO_2.

TERUHIKO OCHIAI & YUSUKE MATSUDA
Department of Chemistry, Kwansei-Gakuin Univ., Nishinomiya 662-8501.

When wild-type cells of *Chlorella ellipsoidea* are grown in high CO_2, the expression of the inorganic carbon concentrating mechanism (CCM) is largely repressed. The CCM is expressed maximally after cells are transfered from high-CO_2 to low CO_2, which confers the cells high photosynthetic affinity for dissolved inorganic carbon (DIC). It was suggested that the repression occurs in response directly to extracellular [CO_2] in *C. ellipsoidea*. This hypothesis is supported by the isolation of the CO_2-insensitive mutants of *C. ellipsoidea* induced by x-rays or N-ethyl N-nitrosourea (ENU). Mutants grown in high-CO_2 exhibited high affinity for DIC, which resembled that of wild-type cells grown in air. In the present study, effects of high CO_2 on cells with maximum CCM were examined and compared between wild-type cells and mutants. Pmax, affinity for DIC, and the rate of growth were measured during acclimation of air-grown cells to high CO_2. The results showed that Pmax reduced temporarily to about 50% and 70% initial values in wild type and mutants, respectively. However, the duration of such Pmax reduction occurred was only for one day in mutants whereas for 2days in wild type. Growth was found to stop for 2days in both cells. Affinity for DIC in wild-type cells started decreasing in 5 days of acclimation and continued to decrease for the next 4days, whereas such decrease in affinity was not observed in mutants. Given these, it is postulated that the CO_2-insensitive mutants possess higher capacity to acclimate to high CO_2 than that of wild-type cells, despite of the continuous operation of maximum CCM under high CO_2 in mutants.

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Genes expressed in relation with the sensitivity to the ambient CO_2 in the green alga, *Chlorella ellipsoidea*.

Dan SATO, Goro HIROZUMI, Yusuke MATSUDA; Department of Chemistry, Kwansei-Gakuin Univ. Nishinomiya. Hyougo 662-8501

Microscopic green algae are known to possess the ability to accumulate dissolved inorganic carbon (DIC) as substrates for photosynthesis from the surrounding media, when grown in limiting-CO_2 concentrations. This mechanism is termed as inorganic-carbon concentrating mechanism (CCM). It has been shown at physiological bases that the CCM in the green alga, *Chlorella ellipsoidea* is repressed in response directly to high [CO_2] in the media and hence derepressed under CO_2-limiting condition. This interpretation is further supported by the isolation of CO_2-insensitive mutants of this alga in which CCM is not repressed even under high [CO_2]. Molecular mechanisms behind the sensitivity of microalgae to changes in the ambient [CO_2] are largely unknown. CO_2-insensitive mutants may provide us with a number of clues to study on this point.

In this study, wild type or mutant-specific cDNA libraries were constructed by cDNA-subtraction method. Results from sequencing and partial characterization of these libraries will be reported.